

MITOSIS

IN

NOCTILUCA MILIARIS

AND ITS

BEARING ON THE NUCLEAR RELATIONS
OF THE PROTOZOA AND
METAZOA

BY

GARY N. CALKINS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
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COLUMBIA UNIVERSITY



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GARY N. CALKINS.

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IN view of the probable origin of the Metazoa from the colony-forming Protozoa, many observers have attempted, more or less definitely, to trace the phylogenetic development of special parts of metazoan cells from analogous parts in Protozoa. Prominent among such attempts have been those of Bütschli ('91), Heidenhain ('94), Hertwig ('96), and Lauterborn ('96), and the two last-named writers have shown that, from the least differentiated parts of the most primitive protozoan nuclei, a progressive series may be traced which culminates in *Nocti-*

luca miliaris, where the nucleus and the mitotic figures are quite as complex as in the Metazoa. Ishikawa ('94) had already shown the similarity between *Noctiluca* and the Metazoa in this respect, although his account of mitosis in the former was incomplete. *Noctiluca* holds so important a place in the series, especially in relation to the process of mitosis, that its nuclear division should be fully and accurately known, and it has been my aim during the last two years to clear up, if possible, the points still left obscure.

The wide distribution of *Noctiluca* and the large size of its nuclear elements have made it a favorite subject for research, although it has seldom been studied from a cytological standpoint. Huxley's description ('55) was purely morphological, as were those of De Quatrefages ('50), Krohn ('52), and Busch ('55). Brightwell ('57), describing the external phenomena of cell division, undoubtedly saw the sphere,¹ but called it a secondary nucleus. Cienkowski ('71 and '73) described the external features of spore-formation, but without reference to the nucleus or the accompanying structures. Robin ('78) in his description of the spore-formation seems to have mistaken the sphere for a nucleus, and his accurate illustrations tell more about the nuclear processes of division than the text.

It was Ishikawa ('94) who first gave accurate details of mitosis in *Noctiluca*. His work can best be reviewed by presenting his own summary (pp. 324-326) as follows :

1. The division of the animal is preceded by the loss of the peristome, teeth, and the tentacle, the last of which is not thrown off, as Robin is inclined to think, but is redrawn into the body of the animal. The mouth and the "Staborgan" are, however, always present (Robin).

2. The spore-forming individuals differ from the dividing ones in not possessing the mouth and Staborgan in addition to the organs above mentioned, and by the excessively empty appearance of the cell interior (Cienkowski).

3. The division of the nucleus is always preceded by a concentration of a part of the cytoplasm in the form of a spherical or oval granular body,

¹ I use the term "sphere" to designate the large, clearly outlined cytoplasmic mass which is probably to be identified as an attraction sphere. During the prophase of mitosis it divides and forms an amphiaster with connecting central-spindle fibers, and in the anaphase a centrosome is found within it.

mostly close to the nucleus. This is the archoplasm or kinetic center of division, and corresponds most probably to the "Nebenkern" of Von la Valette St. George.

4. In living animals at the stage of (3), the nucleus appears more or less homogeneous and transparent, and is not so distinctly to be seen as the archoplasm. But treated with reagents the chromosomes come into view distinctly.

5. Each chromosome consists of a row of disc-shaped microsomes irregularly scattered in the nucleoplasm. The number of the chromosomes is not clear, but in most cases has been counted to be ten.

6. The chromatin substance of each of the microsome-discs collects at the periphery and forms a microsome-ring.

7. In the nucleus of a dividing animal, each microsome-ring splits into half-rings, thus dividing a chromosome in halves, while in that of the spore-forming animals two successive divisions of a microsome-ring take place, so that a single chromosome is directly divided into four daughter ones.

8. The chromosomes collect on the side of the nucleus which is nearest to the archoplasm and spread out towards the other pole. The pole where the archoplasm lies thus corresponds to Rabl's "Polfeld," and the other pole to his "Gegenpol."

9. The archoplasm divides and forms a very large spindle which first lies tangential to the surface of the nucleus. This division of the archoplasm is succeeded by the separation of the chromosomes into two groups, each attracted (?) by its respective archoplasm.

10. The archoplasmic spindle thus formed pushes in the nuclear wall on which it lies, and the nucleus assumes in consequence a half-ring form.

11. By the separation of the archoplasms a spindle is produced which in all essential characters appears like the form known as the "dyaster stage," with a large archoplasmic mass at each end of the spindle.

12. The fibers of this spindle are therefore continuous from one pole to the other, and, lying outside the nuclear wall, become in no way connected with the chromosomes. But there is seen at this stage another set of fibers running from the center of the archoplasm to the polar ends of the chromosomes. This structure of the spindle corresponds exactly with that of the spermatocyte of *Salamandra maculata*, as investigated by Hermann, with only the difference of the persistence of the nuclear wall in *Noctiluca*, and the necessary modification in consequence of this fact. The optical appearance of these two kinds of fibers is different, just as in *Salamandra*.

13. Besides these two sets of fibers, the Verbindungsfaden are clearly to be recognized extending between the separating chromosomes.

14. The central-spindle fibers originate from the archoplasm, the radial fibers, probably from both the cyto- and nucleoplasms, and the Verbindungsfaden from the linin substance.

15. In the spore-buds the archoplasm is to be seen lying close to the nucleus up to the time of full development of the spore, just before its

detachment from the mother-animal, and a part of it becomes transformed into the flagellum, just as in many vegetable swarm-spores, as Strasburger shows.

16. In the center of the archoplasm is generally seen a centrosome, which often shows a dumb-bell form. Sometimes, however, two centrosomes are found in the archoplasm of the spore-forming cells. In many cases, again, there is found in the center of the archoplasm a number of small oval rod-shaped or curved bodies staining exactly like centrosomes, instead of one or two centrosomes. These may represent the group of centrosomes of Heidenhain.

17. The origin and the fate of the centrosome are not known. In a few instances it appears to be formed from the nucleus.¹

Section 5 is rather obscurely worded, but, from the detailed description given elsewhere, it appears that the chromosomes, which are of various lengths, consist of irregular rows of microsomes lying scattered throughout the nucleus. "The chromosomes, except a few, do not seem to lie in definite order, but to be scattered more or less irregularly in the nucleus."

In the descriptive part of Ishikawa's paper section 7 is stated in a more conservative manner: "While the chromosomes of the nuclei of the dividing individual are represented by a double row of microsomes, those of the nuclei of the spore-forming individuals appear to consist of four rows." He explains this difference as follows: "In division the nucleus has to divide only once, and hence the chromosomes require only once to divide, while in the spore-formation divisions of the nucleus take place rapidly one after the other, and two divisions take place almost simultaneously" (*l. c.*, p. 304).

It may be pointed out, however, that in spore-forming individuals the nuclei continue to divide rapidly and without intervening resting periods, until there are from three to five hundred descendants of the original nucleus. What reason, therefore, can there be for quadruple division of the original chromosomes?

The details regarding formation of the nuclear plate and the separation of the chromosomes are not given.

¹ The killing agents used by Ishikawa were Flemming's stronger solution, micro-acetic, and acetic acids. The material was stained with Böhmer's haematoxylin, acid fuchsine, methylin blue, and methylin green.

Ishikawa speaks of "radial-fibers" (section 12), but as these are analogous in every way to similar fibers in ordinary mitosis, I see no reason for adopting this term in preference to the current name "mantle-fibers."

The present paper deals almost exclusively with the points touched upon by Ishikawa in sections 3-14, 16, and 17. The details of chromosome-formation are found to differ considerably from Ishikawa's account, and for the first time the formation of the nuclear plate in *Noctiluca* is described. Ishikawa's observations in regard to the sphere and centrosome are, in the main, confirmed.

Before giving the results of my observations I wish to express my obligation to Professor Wilson, not only for the material upon which most of my work was done, but also for his kind advice and unceasing interest.

I. MATERIAL AND METHODS.

The material for my work was collected partly by Professor Wilson at Beaufort, N. C., in June, 1895, and partly by myself at Port Townsend, Puget Sound, in July, 1896. In the latter place it was found in great abundance, often covering hundreds of square yards, its presence indicated by brilliant phosphorescence by night, or in daylight, when wind and tide were favorable, by patches of brilliant orange.¹ They were collected early in the morning and preserved at intervals during the day and succeeding night, and all stages of vegetation, division, and spore-formation were thus secured. Five different killing agents were used, *viz.*, corrosive sublimate (saturated in normal salt solution), sublimate-acetic (10 per cent acetic), picro-acetic (Boveri's formula, with 1 per cent acetic for the Beaufort material, saturated picric with 5 per cent acetic for that from Puget Sound), Hermann's fluid, and Flemming's stronger solution.

The material was prepared for study either by mounting *in toto* or by imbedding in paraffin and sectioning. Considerable difficulty was experienced in handling such small objects until the following method was devised. The alcohol containing *Noctiluca* was poured into a glass cylinder two inches long and one-half an inch in diameter, covered at the lower end with bolting cloth of such fineness that the *Noctiluca* were held back while the alcohol passed through. They were then readily handled by car-

¹ *Noctiluca* was observed and collected in Alaskan waters as far north as Juneau and Sitka, and even in Glacier Bay, where the water is at a constant low temperature. The water of Puget Sound is deep and cold (about 51° at all depths), but at Sitka it is shallow and perceptibly warmer.

rying the tube from one staining pot to another. In all cases they were cleared in xylol and mounted in balsam. For sectioning, either large quantities of *Noctiluca* were imbedded in bulk, or individuals were selected and imbedded separately. In most cases the latter method gave the most satisfactory results, especially for dividing nuclei, where it was often desirable to section in definite planes. Heidenhain's iron haematoxylin, the Biondi-Ehrlich mixture, and Reinke's modification of the Flemming triple method gave the best results in staining. Bordeaux red, orange, and eosin were used as secondary stains with the iron haematoxylin.

II. OBSERVATIONS.

A. THE RESTING NUCLEUS.

The nuclei of *Noctiluca* belong to the so-called vesiculate type. They are spherical, oval, or elliptical in shape, and vary considerably in size, the largest measuring about 50μ in diameter (Fig. 6), the smallest about 30μ , while those in the various stages of spore-formation are still smaller. They are always enclosed by a distinct, often thick, membrane, which, although it disappears at certain regions during mitosis, is, as a whole, retained through all phases of nuclear change.

The interior of the nucleus consists mainly of two distinct substances. One of these is granular and stains with acid dyes, while it is so abundant that it gives a massive appearance to the nucleus. The staining reactions of this substance show it to be the same, probably, as the "oxychromatin" described by Heidenhain ('94) in the nuclei of leucocytes. Unlike the latter, however, the oxychromatin granules in *Noctiluca* appear to be isolated bodies of large size and not suspended in a colorless network, the "linin" of Heidenhain. The other substance stains intensely green with the Biondi-Ehrlich mixture and represents the "chromatin" of Flemming ('80) or the "basichromatin" of Heidenhain. In the resting nucleus the basichromatin is invariably collected in from eight to eleven, or more, great chromatin reservoirs which, for the sake of brevity, may be called the karyosomes in place of the earlier and more misleading term, — nucleoli. By this use of the term, however, it must not be understood that the karyosomes are local thickenings of a general basichromatic reticulum. There is no such

network in *Noctiluca*, and "karyosomes" here represent the entire chromatic reticulum of metazoan cells. Karyosomes are characteristic of many Protozoa, and the nuclei containing them are classed together by Gruber ('84) as the "vesiculate" type ("Bläschenförmige"). In all such nuclei a membrane, nuclear ground substance, or "sap," and one or more central granules of chromatin can be distinguished.

In *Noctiluca* the two nuclear substances differ widely in their affinity for dyes, the oxychromatin being stained a clear and intense red by the Biondi-Ehrlich mixture, while the basichromatin is stained a brilliant green. This difference in color is so marked, and the limits of the two substances so distinct, that the smallest particles of green basichromatin can be easily detected on the red background of oxychromatin. This stain is, therefore, invaluable in following the chromatin changes during nuclear activity.

In addition to the basichromatin and oxychromatin, a small spherical body (x) surrounded by a hyaline area (3, 4, 6, and 7) is usually to be found in the nucleus. With iron haematoxylin and orange it takes an even more brilliant haematoxylin stain than the chromatin, remaining black or blue while the karyosomes are a deep gray. I have not succeeded in staining it successfully with the Biondi-Ehrlich mixture. In most resting nuclei only one such body is present. From the subsequent changes in the nucleus, and from analogous bodies in other Protozoa, there is some reason to regard this corpuscle as the centrosome.

B. THE SPHERE IN THE RESTING CELL.

In the cytoplasm, outside of the resting nucleus, lies a large cytoplasmic mass which was first pictured by Allman ('72) and later described by Robin ('78) as a nucleus. From its relation to the nucleus and its behavior during cell division this cytoplasmic mass may be called the "attraction-sphere" or simply the "sphere," although in its resting stage no centrosome can be found in it. It is often as large as or larger than the nucleus (Figs. 1 and 8), but differs in appearance at different stages. It

is always in contact with the nucleus, sometimes having the form of a sphere, but more often that of an irregular mass with a darker peripheral and a lighter central portion. The latter appearance is caused by the accumulation of microsomes around the periphery, and by their absence in the central portion. In specimens fixed with Hermann's or Flemming's fluid the sphere is seen to have pseudopodia-like processes extending from it into the surrounding cytoplasm and passing imperceptibly into the reticulum (Fig. 11).

In addition to the microsomes in the sphere other deeply staining, but larger, granules are frequently seen. Sometimes only one of these can be found ; again they are quite numerous, even forming a small group of deeply staining bodies. Similar granules are also found in the cytoplasm, distributed throughout the reticulum. These granules are often strikingly similar to centrosomes and might easily be mistaken for them. Careful comparison of many spheres, however, both in sections and in total preparations, shows that they cannot be centrosomes. Their position is often eccentric (Fig. 5), sometimes even in the periphery of the sphere, while the presence of similar granules in the cytoplasm shows that they cannot be "centrosomes" in Heidenhain's sense. It is not improbable that the centrosomes described by Ishikawa, which were found sometimes single, sometimes double, and sometimes in groups, were cytoplasmic granules of this kind. Ishikawa apparently saw the "sphere" only during division : "The archoplasm, as we have stated, comes to be first seen at the stage a little before the division of the spore-formation" (*l. c.*). My observations, however, leave no doubt that it persists as an extra-nuclear mass throughout all periods.

C. THE NUCLEUS DURING DIVISION.

The phenomena of nuclear division in *Noctiluca* are so complicated and apparently so different from typical mitosis in the Metazoa that the following general summary will help to make the details more clear.

During the early stages of nuclear activity the sphere divides into two similar halves connected by fibers which here, as in

some Metazoa, may be called the "central-spindle" (Figs. 9 and 11). The central-spindle then sinks into the nucleus which forms a trough to receive it (Fig. 13). The trough deepens and the lips of the nucleus approach each other over the central-spindle until the latter finally occupies the position α of the Fig. C, the daughter-spheres lying partly in the groove and partly exposed at the ends (Fig. 11). The nucleus then divides in a plane at right angles to the central-spindle, each half being accompanied by one of the daughter-spheres. The anaphase-stage presents the well-known striated appearance of protozoan mitotic figures — the striations being formed by the daughter-chromosomes which are directed towards the separating spheres (Fig. 18). In the final stage of division the daughter-nuclei become completely separated, the furrow in which the central-spindle had lain becomes obliterated, and the sphere resumes its normal appearance in the resting cell.

In spore-forming divisions the process is in the main the same, but the mitoses follow each other in quick succession and without intervening resting stages. Here the daughter-chromosomes of an anaphase without further change form the chromosomes of the ensuing prophase. The spheres again divide and the process is continued until the original nucleus is divided into as many parts as there will be spores (from 300 to 600).

1. *The Chromatin.*

a. Prophase. — Nuclear division is preceded by a concentration of the cytoplasmic microsomes in the sphere. The hyaline area disappears, the entire mass diminishes in size and becomes more homogeneous, and by these changes the sphere becomes more dense and more clearly defined, so as to be even more conspicuous than the nucleus. It was probably this stage which misled Brightwell ('57) and Robin ('78) into mistaking it for the nucleus.

Within the nucleus, meantime, the large basichromatic karyosomes gradually break up into smaller pieces, apparently in the same way as the chromatin in *Actinosphaerium* according to Gruber's ('83) and Brauer's ('94, '94a) descriptions. The disintegration of the karyosomes seems to be accomplished by a

progressive process of division, and from a study of the various modes of aggregation of the chromatin fragments in different nuclei, it appears that they divide first into two nearly equal parts, these into four, the four into eight, etc. In most cases the parts thus formed soon become scattered so that it is almost impossible to follow them ; but in some favorable cases the parts remain in groups in the places occupied by the original karyosomes until as late as the eight-part stage (Fig. 4). I have never been able to follow the fragmentation beyond this stage. The chromatin-granules thus formed leave their original positions and become concentrated at the side of the nucleus which lies away from the sphere (Fig. 5). But even here it can be made out that the larger pieces continue their fragmentation into smaller and smaller portions. The final result of this disintegration is the formation of a great number of minute chromatin granules similar to those of the nucleus of *Actinosphaerium* (Brauer).

As they break up, the chromatin masses often, but apparently not always, give rise to beaded fibers which have no definite arrangement in the nucleus (Figs. 6, 7, 12). Ishikawa ('94) in his second paper describes these fibers as chromosomes and pictures them in his Figs. 33, 38, 40, 44, and 47. He thought they were formed from the parts ("microsome-discs") of the disintegrated nucleoli (karyosomes) which were put together "one after the other like chains of mammalian blood corpuscles." I am convinced, however, that although these fibers appear in the manner described by Ishikawa, they are not the actual chromosomes. They do not pass directly into the nuclear plate, nor do they split longitudinally. This stage is a prophase of division, as shown by the condition of the sphere (Fig. 12). It is antecedent to the stage of the nuclear plate, and later than that of the resting nucleus. It must, therefore, correspond to the spireme-stage of mitosis, though it cannot be interpreted as a monospireme, for in total preparations the ends of the figures are in full view and are in no way connected with each other (Figs. 6, 7).

After the above changes the granules which compose the fibers of the spireme break down into still more minute

granules — the ultimate chromatin elements (Figs. 9, 10, and 36). These are so fine that one can distinguish them only after the most careful differential staining. At first they are distributed equally throughout the entire nucleus, but even before all of the chromatin of the karyosomes is converted into chromomeres, those which have already formed begin to collect in lines extending from the side of the nucleus which lies nearest the sphere, towards the opposite side (Figs. 9, 10, 32, 35, and 36). These lines of granules are the beginnings of the chromosomes. The Biondi-Ehrlich solution and the iron haematoxylin with orange G shows them best; the bright lines of chromatin granules show distinctly in contrast to the broad lines of oxychromatin lying between them. In sections stained by the first method (Fig. 10)¹ the minute green chromatin elements can be traced through all stages of chromosome-formation, from isolated granules of incredibly small size, distributed throughout the nucleus, to the compact chromosomes directed towards the sphere. The chromosomes next increase in thickness at the peripheral ends, probably by aggregation of the granules, and taper towards the center of the nucleus until at the inner end the threads are no thicker than the single granules (Fig. 11). The tapering of the chromosomes is, however, but a passing phase; later they gradually thicken throughout their entire length and finally become of uniform thickness (Fig. 38).

Throughout all stages of nuclear activity there is no evidence to show that oxychromatin is being changed into basichromatin or *vice versa*. The chromatin granules are derived from the karyosomes, and at all stages can be distinguished from the oxychromatin granules by their intense green chromatin stain. The oxychromatin granules, meantime, do not change in size or in staining reaction. They are many times larger than the chromomeres, and during chromosome-formation they are arranged in lines parallel with the chromosomes (Figs. 9, 32, 36).

¹ These minute granules are difficult to represent in black and white and Fig. 10 represents but poorly the actual preparation. The intense black dots distributed throughout the nucleus and among the larger granules of oxychromatin give a fairly accurate picture.

When the latter are completely formed, these granules fill the remainder of the nucleus, the basichromatin of the chromosomes occupying no more space apparently than when in the form of karyosomes.

b. Metaphase.—At this period the chromosomes are single, thick rods or fibers of chromatin and distinctly granular, although each granule is much larger than one of the original elements. Shortly afterwards, however, the chromosomes become double, consisting then of two rows of granules formed by a longitudinal cleft down the middle line (Fig. 38). The main part of the chromosomes does not lie in the center of the nucleus as usual in other Protozoa (*Actinosphaerium*, *Euglypha*, etc.). On the contrary, the chromosomes lie in a linear group with their ends close against the membrane of the nucleus at the side nearest the sphere (Figs. 8, 11, 26). At this stage, or even before (Figs. 12 and 14), the nucleus begins to elongate in a direction which for convenience may be called the "primary axis," the ends of the chromosomes being arranged in the median line of the nucleus, that is, along the primary axis (Fig. 14). The nucleus thus assumes the appearance of a cylinder with rounded ends, the chromosomes extending from the margin nearest the sphere towards the opposite side (Fig. 14). This stage is rarely seen, however, for the elongate nucleus soon bends around the central-spindle to form a C-like figure (Figs. 12, 13). In many cases the curvature along the primary axis and the elongation of the nucleus are simultaneous and not separate actions. The curvature continues until the extremities of the primary axis become more or less closely approximated (Fig. 13).

By the curvature of the nucleus the chromosomes are carried around the central-spindle until they form an incomplete ring. They become focused at the point *x* of the Fig. C, which is formed by the curvature of the nucleus. The axis in which the central-spindle lies may be called the "secondary axis" (Fig. 13).

c. Anaphase.—The structure of the chromosomes in the nuclear plate and their subsequent changes can be studied only in sections. These may be cut either transversely or longitu-

dinally, *i.e.*, in planes which pass through the secondary or the primary axis. In transverse section the chromosomes are thick, double fibers which tend to converge in the median line and to spread out from here like the ribs of a fan (Figs. 22, 26). At the proximal ends they are so compact that it is difficult to make out the double nature, but towards the middle the parts become more separated, and at the distal ends the granules in the chromosomes can hardly be distinguished from the remaining chromatin elements, which are still free in the nucleus.

Separation of the daughter-chromosomes begins at the proximal ends, *i.e.*, at the ends nearest the spindle. Each chromosome splits down the line of original longitudinal cleavage (Fig. 27, *A*), and the parts move in opposite directions, proximal ends first (Figs. 28, 29). Fig. 38 represents a section through the primary axis, showing the chromosomes in the nuclear plate in the metaphase. Fig. 27 shows the same stage in transverse section. In Fig. 27 the chromosomes have just begun to separate (*A*), while in Figs. 28, 29 the division is further advanced. During the separation which follows, some of the daughter-chromosomes, moving in opposite directions, pass each other, and a curious crossed appearance results (Figs. 28, 29). Fig. 30 is from a vertical section through the secondary axis, showing an anaphase; the chromosomes are turned in opposite directions towards the spheres. Fig. 31 represents the same stage in a section cut horizontally through the secondary axis.

As the daughter-chromosomes separate, the nucleus again elongates, but in a direction at right angles to that of its original elongation, *i.e.*, in the direction of the secondary axis (Figs. 5-20, 30). During this secondary elongation the nuclear lips are pressed more closely together above the central spindle until in some cases a mere slit is all that is left of the former opening (Fig. 17).

d. Telophase. — The description of nuclear division given above may apply equally to vegetative division or to that occurring in spore-formation. The telophase is, however, essentially different in the two cases. In the former, after the chromosomes are completely separated at the distal ends, the nucleus

itself begins to divide. At first this is indicated by a slight depression in the center of the nucleus (Fig. 20). The daughter-nuclei then move further apart until the connecting-piece is reduced to a mere thread, which soon breaks. The membrane reforms, and all traces of the groove are obliterated as each daughter-nucleus finally rounds out. The chromatin of the daughter-chromosomes fuses to form again the great karyosomes of the resting nucleus (Fig. 25), probably by a reversal of the process of disintegration described above. The distal elements of the daughter-chromosomes are the first to disappear, while the thicker parts remain as the last evidence of division (Fig. 25). In some instances the karyosomes begin to form before the daughter-nuclei are separated, as in Fig. 20.

In the spore-forming divisions, on the other hand, which follow each other in quick succession, the chromosome-structure is not altered, and the daughter-chromosomes pass directly into the nuclear plate of the next division figure. This process is repeated nine or ten times until all of the spores are formed. Fig. 21 represents the beginning of a second division before the first is quite complete, and with a relatively large "Verbindungsstück" left between the nuclei. The daughter-chromosomes which are to form the nuclear plate of the second division are single (Fig. 30), and yet just before the second division they are double (Fig. 38). This division must take place while the nuclear plate is forming. Ishikawa states that chromosomes are quadruple in nuclei destined to form spores, but I have never found the chromosomes in the nuclear plate otherwise than single or double. Even were they quadruple in the parent nucleus it would not suffice to explain the eight or nine subsequent divisions which the nuclei undergo before the spores are formed. The elements composing the chromosomes must divide longitudinally before each division of the nucleus. As there is no time for growth of the chromosomes between divisions, the quantity of chromatin in the nucleus is constantly reduced by half, and the nuclei become smaller and smaller.

2. *The Nuclear Membrane.*

In the resting cell the nuclear membrane is comparatively distinct and thick, but in the active nucleus it becomes very much thinner and more plastic. Except in one region it does not disappear during mitosis, but persists as a permanent portion of the nucleus. In one region, however, it does disappear during mitosis. When the nuclear plate is formed and the chromosomes are ready for division, it disappears in the region between the nuclear plate and the central-spindle. The nuclear plate is thus left as a free ring around the central spindle (Figs. 13, 29, 31). As the daughter-chromosomes separate, the membrane is reformed between them (Fig. 30), but it always remains broken in the region between the daughter-chromosomes and the spheres. The position of the gap which is thus formed in the membrane can be clearly seen from Fig. 31. Here the membrane is complete in all places except where the mantle-fibers pass from the centrosomes to the chromosomes. During the telophase the membrane reforms in the broken places and again becomes continuous.

The above-described gap in the membrane during mitosis is strikingly similar to a temporary stage in metazoan mitosis which has been described by many observers. In all such cases the nuclear membrane disappears first at the poles in front of the developing spindle-fibers. In some instances (*e.g.*, in *Thalassema* Griffin ('96)) the membrane persists in this stage for some time, and only at a late period in mitosis does it completely disappear.

3. *The Sphere.*

We may now consider the history of the sphere more in detail. As previously stated, the approach of division is first recognized by the concentration of cytoplasmic microsomes in the sphere. During disintegration of the karyosomes and formation of the chromosomes a structure is formed consisting of two daughter-spheres connected by a "central-spindle," to which, from analogy with metazoan mitoses, the name of amphiasier may be given (Figs. 9, 11). As the nucleus elon-

gates and bends in the plane of the primary axis, the central-spindle sinks into the depression which is thus formed, until it finally occupies the position x in the Fig. C. The spindle thus lies in the secondary axis of the nucleus which encircles it, the spheres alone remaining outside (Figs. 13-15). The nuclear plate is wrapped around the spindle like a ring, the chromosomes lying midway between the two poles. The central-spindle fibers are at first not straight, but run from pole to pole in curved lines (Fig. 11). This curvature is caused by the spheres moving around the nucleus through an arc of 90° , the fibers becoming straight only when the central-spindle finally lies in its definitive position (Fig. 13). In some cases the bend of the central-spindle is much exaggerated until an acute angle is formed. During this time the growing chromosomes are entirely separated from the central-spindle, but when the nuclear plate is complete the intervening membrane disappears, and then, as in the metazoan spindle, the chromosomes lie directly upon the central-spindle. After the preliminary arrangement of the elements of the mitotic figure, the central-spindle undergoes a considerable elongation (Figs. 17-19, 30), while at the same time the nucleus is drawn out in the direction of its secondary axis in the form of a hollow cylinder with a ring of chromosomes at either end (Figs. 17-19). The dumb-bell-shaped amphiaster lies in the hollow with the spheres projecting at either end (Fig. 13). After division of the vegetative nucleus the sphere returns to its resting condition (Fig. 20). It becomes less dense and less homogeneous, and the central part more or less hyaline, while the peripheral portion still retains its granular aspect. The transformation may begin even before the nuclei are completely separated. Such a condition is indicated in Fig. 20, where the daughter-spheres, having lost their densely granular spherical condition, have become more diffuse, and have acquired the characteristic appearance of the resting spheres. On the other hand, after spore-forming divisions, the daughter-spheres do not return to the resting condition. They retain their spherical shape and dense granulation (Figs. 17-19), and soon divide again for the ensuing mitosis. It frequently happens that this secondary division of

the daughter-spheres takes place before the daughter-nuclei of the preceding mitosis are completely separated (Fig. 21). Fig. 21 also shows that the plane of the second division of each sphere is at right angles to the plane of the first division. The same thing is shown in an earlier stage in Fig. 18, where the primary axis of the daughter-nucleus is bending around the sphere; and again in the sections represented in Figs. 24, 39.

In some cases, particularly after fixation with Hermann's fluid, thick but short fibrous processes may be seen passing from the sphere into the cytoplasm (Figs. 9 and 11). These are analogous to the astral rays of metazoan cells, and like the central-spindle fibers they are formed from the substance of the sphere, being made up of granules or microsomes similar to those found in the sphere.

According to the foregoing description, the sphere in *Noctiluca* apparently corresponds to Boveri's archoplasm ('88). It is a persistent cytoplasmic structure, of a definite size and shape in resting and active cells, and appears to consist of a specific substance. Astral rays and central-spindle are formed from its substance, while at certain stages a centrosome lies within it (see p. 21).

The main observations by contemporary writers on the sphere fall into one or other of two groups, according as they agree with Van Beneden's or Boveri's conception of the origin of this structure. Van Beneden thinks that the sphere, while it consists of the same substance, is morphologically different from the cell plasm. Boveri originally considered it (and with it all spindle and astral fibers) as not only different from the cell plasm morphologically, but as composed of an independent substance—the archoplasm. Among botanists the "archoplasm idea" is generally accepted, and is expressed by Strasburger's term "kinoplasma," while among zoölogists this theory has not been generally approved, most observers supporting either Van Beneden's theory or some modification of it. There is, however, the greatest latitude in this support. Many interpret the sphere as formed anew from the cytoplasmic reticulum at each mitosis. (See among others Heidenhain ('94), Reinke ('94), Wilson ('95), Eismond ('95), Kostanecki

('96), v. Erlanger ('97), etc.) Others, however, while regarding the sphere as a differentiation of the cytoplasmic reticulum, find that it is permanent in the cell, dividing by fission like the centrosome. (Van Beneden '87, Meves '95, vom Rath '95.) Here the two views stand rather close together; on the one hand, it is maintained that there is a distinct structure permanent in the cell, but composed of modified cytoplasmic reticulum; on the other hand, it is held that the distinct structure is composed of a distinct substance. The difference here is certainly not great.¹

The fact that in some cases the sphere appears only during mitosis is perhaps the most serious obstacle to Boveri's interpretation. He avoids it, however, by maintaining ('96) that the archoplasm is not necessarily restricted to a definite body, but may exist throughout the cell in the form of minute granules, which during mitosis are attracted around the centrosomes, forming spheres. He now ('97) considers that the eggs of *Ascaris*, upon which his idea was based, differ from other cells in regard to the presence and form of the archoplasm, the general rule being that fibers run directly from the centrosome into the cytoplasm. He also says that with the exception of *Noctiluca* (Ishikawa) he knows of no other form of cell where a solid archoplasmic substance exists around the centrosome.²

Schaudinn's *Paramoeba* must now be included with *Noctiluca*. Kostanecki's ('96) work on *Ascaris* does not sustain Boveri's conception, since he finds that, as in other forms, the spindle-fibers pass directly from the centrosome into the cytoplasm; and his work shows that in regard to archoplasm this form cannot be classed with *Noctiluca* and *Paramoeba*.

¹ In a number of cases the term "archoplasm" has been used to designate portions of the cell which are not obviously homologous with the parts described by Boveri. Thus Foot ('96) calls everything in the cell which stains with Lyons blue "archoplasm," and Moore ('94) apparently restricts the term to the residual spindle-fibers (Nebenkern).

² "Ja, mit Ausnahme von *Noctiluca*, wo das Archoplasma nach Ishikawa genau den Eindruck jener körnigen Substanz des *Ascaris*-Eies macht, wüsste ich keinen anderen Fall zu nennen, wo zunächst im Umkreis des Centrosoms eine dichte körnige Kugel besteht, die sich allmählich in das Strahlensystem umwandelt" (*l. c.*, p. 40).

It appears, therefore, that the archoplasm-theory in its original form is far from being definitely established. Heidenhain ('94, '97), one of the chief opponents of the archoplasm idea, has, however, described a substance connected with the centrosome which is of considerable interest in relation to that theory. In resting cells this substance is described as follows: "Diese Centrankörper sind innerhalb des Microcentrums durch eine Zwischenmasse miteinander verbunden, welche meist recht gut sichtbar ist, und im günstigen Fall durch das Rubin der Präparate stark gefärbt erscheint. Diese Zwischenmasse entspricht ganz genau der Materie der 'primären Centrodosome' beim Leukocyten" ('97, p. 231). It is composed of minute granules, and from it are developed the fibers of the central-spindle which form the secondary "Centrodasmus." This substance would seem to agree to a certain extent with the "archoplasm" of Boveri's theory, and I believe that it may be compared to the substance of the sphere in *Noctiluca*. Like the centrodasmus, the sphere is made up of granules surrounding the centrosome, and during mitosis the central-spindle fibers are formed from it. Like archoplasm, it is a specific substance in the cell, surrounding the centrosome and giving rise to the spindle-fibers. While Boveri's original conception of archoplasm as a distinct substance in the cell may not hold for the Metazoa, it is, I believe, entirely consistent with the facts in Protozoa, as will be shown in the comparative part of this paper.¹

4. *The Mantle-fibers.*

It has been shown above that the fibers of the central-spindle have no connection with the chromosomes, but pass without interruption from pole to pole. In the metaphase, and after the disappearance of the membrane, a second set of spindle-fibers is formed, passing from the sphere to the ends of the chromosomes. These are the mantle-fibers or "radial-fibers" of Ishikawa. He speaks of them as follows: "Of the origin of the mantle-fibers in *Noctiluca*, I can say but a few words,

¹ Cf. p.

since the whole problem remains obscure and requires a thorough study with better methods and optical instruments. In sections given above, these fibers which are formed within the nucleus and probably attached to the chromosomes appear to come into close juxtaposition, but not to be continuous with those without, *i.e.*, those seen within and without the nucleus appear to be different from each other, the former originating from the nucleoplasm and the latter from the cytoplasm, just as Brauer thinks concerning the formation of the spindle-fibers of *Ascaris megalocephala bivalens*" (p. 323). My observations, while not conclusive, show, I believe, that the mantle-fibers (or "radial-fibers" of Ishikawa) are connected with the "nucleoplasm," but lie completely outside of the nucleus in the substance of the sphere. The mantle-fibers in mitotic figures of most Metazoa are usually first visible just before or during the time of disappearance of the nuclear membrane. So it is with *Noctiluca*, but here, as in the first stages of spindle-formation in various eggs, the membrane, instead of disappearing entirely, fades away in one part only.

Much has been written during the last few years on the origin of the spindle-fibers, the main question being whether they are of nuclear or cytoplasmic origin. In the different cells of Metazoa the fibers arise sometimes from the nucleus, sometimes from the cytoplasm. In other cases, *i.e.*, when the mitotic figure contains a central-spindle and mantle-fibers, they arise from both nucleus and cytoplasm, in which case the central-spindle usually comes from the cytoplasm (Hermann ('91), Meves ('96), Flemming ('95), Heidenhain ('94), Ishikawa ('94)).¹ In many cases, however, no central-spindle can be distinguished, and in such forms the fibers sometimes arise in the cytoplasm (Strasburger ('92, '97), Mottier ('97), Osterhaut ('97), and botanists in general; Griffin ('96), Wheeler ('95), Mead ('97), etc.), or, in many cases, from the nucleus (Weismann and Ishikawa ('89), Brauer ('93, '94), Rückert ('94), Korschelt ('95), Wilson ('96), v. Erlanger ('97)).

I have already shown that the fibers of the central-spindle

¹ An exception is found in *Ascaris megalocephala univalens* where the central-spindle is intra-nuclear (Brauer ('93)).

in *Noctiluca* are formed from the substance of the sphere, or archoplasm. The mantle-fibers are probably nuclear in origin, as indicated by Ishikawa. They are focused in the centrosome and connect with the chromosomes. The microsomes composing them are at first only loosely strung together (Fig. 38), but later they become more closely packed, forming solid fibers (Figs. 30-39). Since the chromosomes lie in a ring around the central-spindle the mantle-fibers also surround it, and thus complete the similarity to some mitotic figures in Metazoa (Figs. 13, 28, and 29).

When fully formed, the mantle-fibers seem to be stiff filaments of constant length and thickness. They do not shorten as mitosis progresses, nor do they become thicker, but in the telophase they appear the same as in the earlier anaphase (Fig. 24).

5. *The Centrosome.*

It is with some hesitation that I undertake a description of the centrosomes in *Noctiluca*, for they are so minute and are accompanied by so many large cytoplasmic granules that, except in mitosis, their identification is not only difficult, but sometimes impossible. I am certain that Ishikawa, in many cases at least, mistook some of the cytoplasmic granules for centrosomes, for I have been entirely unable to find centrosomes of the size and form described by him. Nevertheless, his general conclusion that a centrosome in *Noctiluca* does exist is correct; for its identification when connected with the mantle-fibers presents no difficulty whatever (Figs. 23, 24, 28, and 38). In regard to its origin Ishikawa had little to say, but he surmised that, in some cases at least, it came from the nucleus.

While fully appreciating the difficulties in the way of determining the origin of a body so minute and so easily confounded with other granules, there are, nevertheless, several phenomena connected with the prophase of nuclear activity which give, I believe, a substantial basis for the conclusion that the centrosome is a minute granule lying inside the nucleus of the resting cell, and that it migrates out into the attraction-sphere during division.

In the metaphase the centrosome can easily be identified in all nuclei, whether undergoing vegetative or spore-forming division, and in the latter it can be distinguished at all stages. In spore-forming mitoses it divides early in the anaphase so that single centrosomes are rarely seen. I have not observed its division in the anaphase of vegetative mitoses; it probably remains undivided until the prophase of the next division. In the metaphase the double centrosome lies in the inside of the sphere, but usually in an eccentric position (Fig. 38). In Fig. 38 (a mitosis in a spore-forming cell) the two daughter-centrosomes lie close together and separation would take place later in a direction at right angles to the plane of the paper. The preceding division is just finished, and the daughter-centrosomes have divided for the ensuing mitosis.

The daughter-centrosomes divide in the early anaphase, after which they lie more nearly in the center of the sphere (Figs. 28 and 31), and, as a rule, they remain near the center until the division is complete (Figs. 23 and 39). They may lie eccentricly, however, even in the telophase (Fig. 24), but in this case it is away from the nucleus. Except for the mantle-fibers, the centrosome is in no way the center of a radial system; the sphere lies around it as an inert undifferentiated mass.

The centrosome appears to vary somewhat in form and in size. In some cases it is comparatively large and round (Fig. 24); in others it is round but much smaller (Figs. 23, 39); while in still other cases it is irregular in outline or even triangular (Fig. 38).

In the resting cytoplasm I have failed completely to find a centrosome in the sphere. In the resting nucleus, however, there is a minute body which stains intensely with the iron haematoxylin and which differs considerably in appearance from chromatin granules (see p. 7). This body disappears during the chromosome-formation and is not seen again until the nucleus reforms after division (compare Figs. 3, 4, 6, 7 with 8, 9, 10, 11, etc., where chromosome-formation has begun). It can always be distinguished from the chromatin granules by its luster and small spherical form. It takes no part in spireme-formation, but remains a separate element (Figs. 6 and 7). At the begin-

ning of nuclear activity it generally lies in that half of the nucleus which is nearest the sphere, and often quite close to it. I have been unable to trace it further than this, when it disappears. At about the same time — *i.e.*, at the beginning of chromosome-formation — the nuclear membrane, lying just below the sphere, shows distinct undulations or wrinkles, while it is intact and uniform at all other points. These can be seen only in sections, but I have found them in all sections of nuclei in this stage (Figs. 32–36), while in sections of nuclei in resting stages the membrane is as smooth at this point as at all others. At the points where the undulations appear, spaces are formed by the elevation of the membrane. These spaces are, as a rule, free from the basichromatin and oxychromatin of the nucleus (Figs. 32 and 33). In numerous cases, however, two minute, deeply staining granules were found in the spaces thus formed. The granules were always found in pairs, and in many instances no other granules could be seen (Figs. 32–34). In other cases, and, I am obliged to admit, in the majority of cases, other granules were found. The latter always had the appearance of chromatin, and it is not surprising that chromatin granules should be found here, for the nucleus at this stage is full of them. The two granules in question, however, have a distinct brilliancy, or luster, and usually have a definite position in relation to the elevations of the membranes (Figs. 32 and 33). Fig. 34 represents a section where the two granules lie just outside of the nuclear margin, which is distinctly indented at this point, and where the nuclear membrane could scarcely be distinguished. They are not in the substance of the sphere, but lie in a hyaline space between it and the nucleus. A similar stage is represented in Fig. 35. In this case only two other granules were visible, and these are represented on the left of the figure near the membrane. They are similar in form and appearance to other cytoplasmic granules found in the protoplasm of *Noctiluca*. Finally, in Fig. 36, the two granules lie in the sphere just outside of the nuclear membrane and the latter is rapidly assuming its regular contour. Other granules are pictured in the cytoplasm and in the sphere.

R. Hertwig ('96) doubts Ishikawa's account of the centrosome in *Noctiluca*, and, in accordance with his theory regarding this body, he would probably consider the sphere in *Noctiluca* as an enlarged centrosome. He also regards the centrosphere in the sea-urchin egg as an enlarged centrosome, in opposition to Boveri ('95), Hill ('95), and Kostanecki ('96), who have found centrosomes within it.¹ From his experiments with strychnine on developing eggs he also concludes that the centrosome may have the form of pole-plates, as in some Protozoa. He supposes the centrosome to be, originally, an intra-nuclear structure (as in *Euglena*, *Spirochona*, etc.), which becomes extra-nuclear in *Noctiluca*, *Paramoeba*, and the majority of Metazoa. The intra-nuclear pole-plates of most Protozoa, the sphere in *Noctiluca* and *Paramoeba*, and the sphere in Echinoderm eggs are, therefore, regarded by Hertwig as homologous structures, each of which he would call the centrosome. At first sight this is a most plausible theory, but many indubitable facts stand in its way. Boveri, Hill, and Kostanecki have found centrosomes in the sphere of sea-urchin eggs; Balbiani ('95) has described less substantially a centrosome in the pole-plate of *Spirochona*; and Ishikawa demonstrated the centrosome within the sphere of *Noctiluca*, an observation which I can fully confirm.²

As there is no trace of the centrosome in the sphere during nuclear rest, the conclusion is apparent that its presence here during nuclear activity can be accounted for either by formation *de novo* or by migration from some other part of the cell. This alternative involves a question in cellular biology which is far from settled — *viz.*, is the centrosome a permanent element of the cell?

In the first place, a number of investigators, especially among botanists, deny entirely the presence of a centrosome in certain dividing cells (Strasburger ('97), Osterhout ('97), Mottier ('97), etc.), while the remarkable observation made by Juel ('97) seems to indicate that the centrosome is not necessary in the formation of a spindle-figure. Juel found that a single chromosome was parted from its fellows and formed a

¹ Wilson also ('97) finds unmistakable centrosomes in *Toxopneustes* and *Arbacia*, which are derived from the middlepiece of the spermatozoa.

² See appendix, p. 49.

separate small cell around itself. It then passed into the resting stage, from which it emerged to form a mitotic-figure in which all of the elements, save centrosomes, were present. In the second place, a number of observers have maintained that, for each mitosis, the centrosome arises *de novo*, as in the well-known theories of Bürger ('92), Watasi ('93), Reinke ('94), and others. The results which Hertwig ('95) and Morgan ('96) obtained by treating the cell with dilute poisons, etc., are evidence in the same direction. Foot ('97) also holds that the centrosome in the first cleavage-spindle of *Allolobophora foetida* is the physiological effect of the spermatozoön upon the cytoplasm of the egg, and not a permanent morphological element brought in by the spermatozoön. To this list must be added a great number of observers who have followed the history of the centrosome up to a certain point in cell changes, after which they found no further trace of it. Finally, many observers have followed the centrosome from one generation to another and maintain it to be a permanent cell organ in accordance with the original view of Boveri ('87) and Van Beneden ('87). Brauer ('93) and the majority of spermatologists have traced the centrosome from generation to generation, in some cases up to a certain definite structure in the mature spermatozoön (Moore ('93), Meves ('96), Hermann ('92), Calkins ('95)), and many observers have followed the centrosome in the same way in the cleavage of the egg (Boveri ('87), Van Beneden ('87), Henneguy ('91), Mead ('95),¹ Griffin ('96)²).

From this review of the subject it is plain that the question of permanency of the centrosome has no immediate prospect of settlement. Repeated examinations of my sections, the evidence of which I have given above, I believe, justifies the conclusion, in a provisional sense at least, that the centrosome of *Noctiluca* is a permanent element; that it exists during the

¹ Mead later ('97) comes to quite an opposite view. He says: "The foregoing observations convince me that the asters and centrosomes in the *Chaetopterus* ovum arise by modification of the cytoplasmic reticulum" (p. 394).

² Wilson ('97) has traced the sperm-centrosome through the first cleavage and into the 2-celled stage in *Arbacia*. Contrary to the view of Doflein ('97), he finds that the centrosome is not represented by the entire middlepiece of the spermatozoön, but that it is contained within the middlepiece which is thrown off as a shell after the spermatozoön enters the egg.

resting stages as a deeply staining granule in the nucleus (see p. 7); that it divides and gives rise to the two granules whose history I have given; that these finally come to lie in the cytoplasmic sphere where they separate as the amphiaster is formed, one going to each daughter-sphere, where mantle-fibers connect them with the chromosomes.

The nuclear origin of the centrosome has been observed in a number of forms. Thus, in all Protozoa hitherto described, with the possible exception of *Euglypha*, *Noctiluca*, and *Parameoeba*, the centrosome, or its equivalent, is intra-nuclear in origin. In the Metazoa the well-known results which Brauer obtained in the case of *Ascaris megalocephala univalens* have not been duplicated for other forms, although a number of observers have been led to think that, as in *Ascaris*, the centrosome is intra-nuclear. Among these may be mentioned Balbiani ('93), Julin ('93), Mathews ('94), and Carnoy and Lebrun ('97). O. and R. Hertwig have long maintained that the centrosome is primitively intra-nuclear, having been differentiated originally from part of the nuclear substance. There is a very important difference, however, between the intra-nuclear centrosome of Metazoa and Protozoa. Both Brauer and Mathews, for example, describe the centrosome as surrounded by a sphere, whereas in *Noctiluca* the sphere lies permanently outside of the nucleus.¹ If my observations are correct, sphere and centrosome in *Noctiluca* must, therefore, have an independent origin. As already indicated, however, there are so many chances for error in tracing the centrosome in *Noctiluca* through resting stages that this conclusion must remain an open question until further research throws more light upon it.²

¹ Mathews's statement of this process is as follows: "At maturation the centrosomes are first accurately to be distinguished as two (at a very early stage apparently as one) deeply staining, small, but distinct and characteristic, granules lying side by side either in the nuclear membrane or immediately without it, and invariably on that part of the vesicle nearest to the surface of the egg. Occasionally one of the granules appears before the other and migrates some distance from the nucleus before the second appears. In cases where they both lie clearly outside of the nucleus, the nuclear membrane is invariably broken behind them" (p. 324).

² Except for its decided staining qualities this intra-nuclear body might be considered a nucleolus and so be used as evidence in support of views of Karsten

D. THE MECHANISM OF MITOSIS IN NOCTILUCA.

The peculiar type of mitosis in *Noctiluca* may perhaps throw some light on the mechanism of mitosis in general. Here the entire absence of astral rays passing from the sphere into the surrounding cytoplasm excludes all contractility hypotheses in so far as they are based upon active physiological contractility of these fibers (Van Beneden, Boveri, Flemming, Reinke, etc.), and the same remark applies to Heidenhain's view that mitosis is affected through elastic tension of the rays. Furthermore, there is no morphological evidence to show that division is affected by contraction of the mantle-fibers (Hermann), for, as shown above, these do not shorten and thicken, but remain the same throughout mitosis. The possibility that their substance is taken up by absorption into the sphere, as Wilson has suggested for *Toxopneustes* ('96), is removed by reason of two facts, *viz.*, the daughter-spheres do not enlarge in the anaphase (spore mitosis) and the mantle-fibers can always be traced to distinct points in the sphere, *i.e.*, to the centrosomes. It must be, therefore, that the separation of the daughter-chromosomes is caused either by an active divergence of the spheres, or by growth of the central-spindle fibers and the consequent passive separation of the spheres (Drüner). The former is improbable because of the entire absence of the antipodal cones or astral rays, leaving the second as the only mechanical hypothesis which agrees with the facts.

My conception of the process is, then, as follows: the central-spindle lying within the ring of chromosomes is advantageously placed for exerting the necessary dividing force. The nuclear membrane disappears and mantle-fibers connect the ends of the chromosomes with centrosomes in the spheres. The central-spindle elongates, causing separation of the spheres; the mantle-fibers, remaining firm, move with the spheres, dragging ('94), Lavdowsky ('94), Carnoy and Lebrun ('97), and others who regard the nucleolus as the seat of the centrosome. On the contrary, it is a body which much more resembles the so-called "nucleolus-centrosome" described by Balbiani in *Spirochona*, and should be, I think, placed with the latter structure as one of the primitive forms of the sphere. See appendix, p. 49.

the ends of the chromosomes with them. As the central-spindle becomes longer, the chromosomes are more and more separated, until finally the distal ends are separated and chromosome division is completed.

R. Hertwig ('95) has already pointed out that this view of the mechanics of mitosis is perfectly consistent with the facts of nuclear division in the Infusoria. No mechanical hypothesis, however, can fully explain the different phenomena involved in the mitosis of *Noctiluca*. The action of the central-spindle cannot explain the first elongation of the nucleus in the primary axis, nor the later ring-form. Neither does it offer any explanation of the forces which cause the in-sinking of the central-spindle into the position of the secondary nuclear axis. The ultimate cause of these phenomena remains unexplained, and there seems to be little doubt that something deeper than mere mechanical force is necessary to explain mitotic activity. This is strikingly confirmed by Juel's observation on the isolated chromosome of *Hemerocallis fulva* and by Boveri's recent ('97) experiments on sea-urchin eggs in which he found that blastomeres are incapable of dividing when chromatin is absent.

III. THE NUCLEAR RELATIONS OF NOCTILUCA TO METAZOA AND PROTOZOA.

A. RELATIONS TO METAZOA.

The similarity of the mitotic-figures of *Noctiluca* to those of the Metazoa has already been indicated by Ishikawa. The comparison can now be carried still further. They agree in the following points: (1) the central-spindle fibers end in spheres which contain centrosomes; (2) the central-spindle occupies a position in the center of the nuclear plate; (3) the chromosomes lie freely around it without an intervening nuclear membrane; (4) the central-spindle fibers are not connected with the chromosomes; (5) mantle-fibers connect the chromosomes with the centrosomes; (6) the chromosomes in *Noctiluca*, like those of the Metazoa, are composed of granules which are at first separate, then unite to form a segmented spireme,

and after division again become disintegrated ; (7) as in the Metazoa the chromosomes divide by longitudinal division ; (8) the centrosomes, finally, are equivalent ; they are the focal points of the mantle-fibers ; they lie in the spheres during activity, and they divide during the anaphase in preparation for the ensuing division. There is some evidence that, as in *Ascaris megalocephala univalens* (Brauer), they come from the nucleus. In only one respect does the mitotic-figure in *Noctiluca* differ from that of the Metazoa—the nuclear membrane does not entirely disappear. In all other respects its description would answer for that of any ordinary metazoan mitotic-figure.

B. RELATIONS TO PROTOZOA.

Mitosis in *Noctiluca* is so similar to that in Metazoa that in itself it throws little light on the origin of the process. The rapid increase of our knowledge of indirect division in the Protozoa has, however, made it possible to draw an accurate comparison between *Noctiluca* and other Protozoa in which the phenomena of mitosis appear in a still simpler form ; and here we find some light on the probable origin of mitosis.

1. *Origin of Chromosomes.*

In many primitive forms of protozoan nuclei, the chromatin is compressed into a single homogeneous sphere (*Uroglena*, *Dinobryon*, *Eudorina*, etc.), and with no indication of “achromatic” substances in the form of “ground-substances” or “nuclear-sap.” In other forms of nuclei closely allied to these, the chromatin in the resting stage is similarly collected into a homogeneous sphere, but it lies imbedded in the nuclear ground-substance, the whole surrounded by a nuclear membrane (*Actinophrys sol*, Gruber ('83), *Heterophrys*, *Acanthocystis*, *Artodiscus*, etc., Penard ('89), and many Flagellates). Penard and Gruber found that in those cases where the chromatin forms a single mass it becomes divided into two, three, or more separate portions. Rhumbler ('90) agrees with Gruber and Penard in such an arrangement of the chromatin in Rhizopods and Heliozoa, and Wolters ('91), Labbè ('97), Clarke

('95), and others find similar results in the Sporozoa. Similarly Schultze ('66) described the nucleus of *Gromia* as variable; one type containing very large granules; another, granules of medium size; and still a third type with very fine granules. Other observers on different forms of Protozoa have given similar descriptions.

From what we now know about the chromatin changes in Protozoa, it is probable that the different types of nuclei which, like *Gromia*, have been described for the same organism are, in reality, developmental stages in preparation for division. Gruber ('83) first showed that in *Actinosphaerium* disintegration of the central chromatin-mass is the earliest indication of mitosis. He found that it first divides into two portions, then into four, later into eight, and so on until a great number of minute chromatin elements fills the nucleus. The nuclear plate is then formed, and division of the nucleus ensues. This observation was confirmed by Hertwig ('84); and Brauer ('95) gave the same general result.¹

In other Protozoa the chromatin is permanently in the form of small chromatin elements. This is the case in *Amoeba proteus*, *Amoeba binucleata*, and *Amoeba crystalligera*, *Euglena viridis*, *Cryptomonas*, and the macronuclei of the Infusoria. From Schaudinn's description ('94) the chromatin elements in *Amoeba crystalligera* do not change during mitosis but are simply separated into equal parts by nuclear division. Similar results were obtained by Blockmann ('94) and Keuten ('95) in *Euglena viridis*.

According to the latter's account, the nucleus of this flagellate consists, as in *Amoeba crystalligera*, of a peripheral ring of elongate chromatin bodies, and a central "nucleolus." In division the nucleolus first elongates while the chromatin is arranged in radial lines. Elongation of the nucleolus continues until the connecting-piece is reduced to a thin fiber. The chromatin is not formed into chromosomes, but is divided into two equal masses,

¹ Brauer's account of the resting nucleus differs in detail from that of Hertwig and Gruber. He showed the nucleus to be similar to that of a metazoan cell, consisting of chromatin in the form of a reticulum and "achromatin" in the form of linin. I have examined many hundred nuclei of *Actinosphaerium* in the vegetative state and after fixation with sublimate acetic and Hermann's fluid, but in none of them could I find the mononucleolate nucleus described by Gruber and Hertwig. The chromatin reticulum was invariably present with from two to four net knots.

and the daughter-chromatin elements gather around the daughter-nucleoli, with connecting or "Zwischenfaden" elements, which form parallel lines from pole to pole. These give the striated appearance which is so characteristic of protozoan mitosis. The daughter-nucleoli finally become totally separated, and the chromatin, which has become massed at the distal ends of the striae, but which still consists of separate units, forms two clumps around them.

In these forms the chromatin appears to be perpetually ready for nuclear division, and the many elements never fuse to form a homogeneous chromatin-mass. Brauer ('95) in describing the formation of the chromatin elements carried the analysis a step further in *Actinosphaerium*.

He found that, as in the metazoan nucleus, the first stage in division is the disappearance of the net knots, the reticulum becoming more distinct. The latter then disintegrates and the nucleus is filled with minute chromatin elements. "Auf einem etwas späteren Stadium ist der ganze Kernraum mit isolirten Körnern, den Chromosomen, erfüllt, deren Zahl nicht zu bestimmen ist" (p. 203). These are not chromosomes, as Brauer states, but elements which fuse later to form the chromosomes extending across the middle of the nucleus. They are next transversely divided, no longitudinal division taking place. It is probable that such an arrangement of the chromatin in the nuclear plate of *Actinosphaerium* represents a primitive stage in chromosome-formation.

The chromatin in metazoan nuclei passes through a similar stage before each mitosis, and similar elements are welded together to form chromosomes of definite shape and size for each species. A long step in the direction of this metazoan condition is taken by *Noctiluca*, where chromatin elements are formed as in *Actinosphaerium*, but become more compactly fused to form chromosomes of a certain distinct character, and where these chromosomes are divided longitudinally.¹

¹ An entirely opposite view of the significance of chromosomes has been maintained by Mitrophanow. According to him, the chromatin in the nucleus of *Collozoum* is a single compact mass: "la chromatine, en forme d'une petite masse arrondie, et l'achromatine, que a l'aspect de deux appendices coniques" (p. 625). He insists that division here is a simplification of mitosis, which, "deviendra claire, si nous considérons la masse de chromatine comme une chromosome unique" (p. 626). This interpretation stands alone in the literature of protozoan mitosis, and instead of simplifying the problem makes it more complex. Mitrophanow's material was fixed with nitric acid and stained with an aqueous solution of safranin, and it seems probable, therefore, that a better technique will give quite different results in *Collozoum*.

A spireme-stage is wanting in the prophase of *Actinosphaerium*, and I have been unable to demonstrate a typical spireme in *Noctiluca*, although, as previously pointed out, the chromatin passes through a stage which simulates the metazoan spireme (Figs. 6, 7, and 12). There are, however, certain Protozoa in which a true spireme seems to occur. Karawaiew ('95) describes spireme-formation in the Radiolarian *Aulocantha scolymantha*. In the resting stage, this nucleus has a large "spongy" nucleolus, consisting of a dense central mass with numerous branches. When preparing for division the "spongy" mass breaks up into threads, until finally the entire chromatin-mass becomes a tightly wound spireme. Sooner or later the spireme-threads undergo a longitudinal division, at the same time becoming granular. Karawaiew saw no nuclear plate, the next stage after the spireme being a late anaphase in which two striped regions were found at the poles. Each stripe consisted of a row of chromatin-granules. The account is by no means complete, and it is probable that more careful examination will show a nuclear plate.

Our acquaintance with the chromatin-changes in the micro-nuclei of ciliates is more satisfactory. The exceedingly dense structure of the micronuclei has led Hertwig and Gruber to class them as "massive nuclei." During division they swell considerably, a change invariably preceded by a spireme-formation (Maupas ('89) and Bütschli ('76)). The spireme gives rise to granular chromatin-threads which as in *Actinosphaerium*, become thicker in the central portion, where they are finally divided by transverse division.

In the great nuclei of Dinoflagellates also, Bütschli ('85) and Lauterborn ('95) have shown that the chromatin-reticulum becomes "increased in size" until a "much-twisted Knäuel" results. After this the chromatin becomes arranged in more or less regular parallel fibers which are divided transversely. Zacharias describes for the same form a much more complex process of mitosis, but his results are denied by Lauterborn and others.

A much more complicated mitosis, including spireme and chromosome-formation, was described by Pfitzner ('86) in the

case of *Opalina ranarum*. Schewiakoff ('88) gives a somewhat similar description of mitosis in *Euglypha alveolata*.

Here the chromatin network of the nucleus gradually becomes thicker, especially in the region of the net knots, until a coarse spireme appears. Threads composed of many small particles — the "chromatin granules" of Pfitzner — are finally formed from the spireme; these are ragged at first, but become homogeneous and smooth, after which they arrange themselves around the periphery of the nucleus. The threads then shorten and become bent into loops, the angle being turned towards the axis of the spindle. At this stage each chromatin-loop is divided longitudinally and the daughter-chromosomes separate. After the formation of a daughter-spireme the nucleus returns to the reticular state.

From this examination of the different changes undergone by the chromatin in the various forms, it is possible to get an idea of the probable development of chromosomes, although the many gaps in the series and the often incomplete observations make it far from conclusive. The most primitive structure would seem to be the mononucleolate forms in which the nucleus has no "cell sap," and where division is possibly "amitotic." An advance is shown in forms where the mononucleolate chromatin-mass breaks up into smaller elements during division, as in some Rhizopods and Heliozoa, and in many Protozoa just before spore-formation. A still higher type is shown in forms where the chromatin exists permanently in the form of small granules (many *Amoebae*, *Euglena*, etc.). In *Euglena* and *Amoeba* these chromatin-granules do not fuse to form definite structures (chromosomes); they simply separate half from half, and they are clearly equivalent to the minute elements, which in other cases are formed by the breaking down of chromatin-masses. The aggregation of chromatin elements into more or less definite chromosomes is shown in a primitive way in *Actinosphaerium* and the micronuclei of Infusoria. In *Noctiluca* the aggregates become more compact, definite, and chromosome-like, while for the first time they are divided longitudinally. A still more metazoan-like chromosome structure is shown by *Euglypha alveolata*, where the chromatin elements are not distributed throughout the nucleus, but unite at once to form a tightly wound thread — the spireme — from which the chromosomes are later derived by transverse division.

These chromosomes, as in *Noctiluca*, divide longitudinally, and daughter-spiremes are formed, after which the chromatin passes again into the resting reticulum. In *Euglypha*, therefore, the chromatin-changes seem to be practically the same as in the Metazoa, and the elements are even more highly differentiated than in *Noctiluca*, for the latter has no chromatin-reticulum nor definite spireme. The aggregation of the chromatin elements after division to form karyosomes in *Noctiluca* is another indication of the more primitive nature of this form. *Noctiluca* and *Euglypha*, therefore, may justly be considered as connecting links, so far as chromatin is concerned, between the conditions in Metazoa and the simplest Protozoa.

2. *Origin of Centrosome and Sphere.*

The origin of the metazoan centrosome and attraction-sphere from simpler elements in Protozoa has been the subject of a number of interesting theories. The most recent and the most important of these have been maintained by Heidenhain ('94), Lauterborn ('96), and Hertwig ('96). According to Heidenhain the "central-spindle," as described by Hermann ('91), is identical with the spindle formed by the micronucleus of the Infusoria after the latter has undergone a loss of chromatin and has acquired a differentiated center — the centrosome. He regards the nucleus of the metazoan cell as derived from the infusorian macronucleus, while the mantle-fibers are new formations. Not only does he compare the nucleus and centrosome with the macro- and micronuclei of Infusoria, but he even makes this comparison the basis of a theory in which he derives the Metazoa from the Infusoria. The comparison of centrosome in Metazoa with the micronucleus is not original with Heidenhain. Bütschli ('91) had already proposed it, and Hertwig and Lauterborn had adopted the same view. Lauterborn's recent attempt to derive the "achromatic" structures of Metazoa from elements in the Protozoa is even more ingenious than that of Heidenhain. The essential difference between the two theories is that in the one case (Heidenhain) the centrosome has been derived directly from the nucleus, while in the other (Lauterborn),

centrosome and micronucleus are supposed to have had a common origin. Lauterborn thinks that centrosome and micronucleus may have had a common ancestor in one of the nuclei of some primitive binucleated Protozoön, and that intermediate stages are represented by certain existing Protozoa. Schaudinn's *Paramoeba* furnishes an hypothetical early stage in differentiation of the centrosome, which there is represented by the Nebenkörper. *Noctiluca* and the diatoms represent a more advanced stage toward the metazoan centrosome. On the other hand, the micronucleus of the Infusoria represents a differentiation of the same primitive nucleus in a different direction.

Hertwig's theory deals more specifically with the development of the "achromatic" parts of the mitotic figure. He expresses it in one paragraph as follows: "Bei den Protozoen finden wir alle Uebergänge von der gewöhnlichen Durchschnürung des Kerns bis zu complicirten Karyokinesen. In vielen Fällen — z. B. den Hauptkernen der Infusorien — ist unzweifelhaft während der Theilung ein achromatisches, dicht mit Chromatinkörnchen beladenes Netzwerk allein der Sitz der treibenden Kräfte; es bilden sich weder Spindelfasern noch Polplatten. Bei *Ceratium hirundinella* ordnet sich das achromatische Kernnetz schon zu Spindelfasern an, auf denen die Chromatinkörnchen gleiten, um auf die Tochterkerne vertheilt zu werden. Einen weiteren Fortschritt macht *Spirochona* durch die Entwicklung von Polplatten. Unzweifelhafte Karyokinesen endlich treffen wir bei *Actinosphaerium*, *Actinophrys*, *Amoeba binucleata* den Nebenkernen der Infusorien. Bei *Paramoeba Eilhardi* und *Noctiluca* scheint sogar der letzte Schritt der Vervollkommnung, die Ausbildung von Centrosomen, gemacht zu werden. Wir würden daher drei verschiedene Ausbildungsstufen in der Entwicklung der Centrosomen aufstellen können: (1) Die achromatische Substanz ist im ruhenden Kern zwar noch gleichmässig vertheilt, liefert aber während der Theilung Polplatten als Aequivalente von Centrosomen. (2) Die achromatische Substanz ist dauernd zu einem intra-nucleären Centrosoma umgebildet. (3) Sie ist zur Bildung eines extra-nucleären Centrosoma aus dem Kern herausgetreten" (pp. 77, 78).

While agreeing with Hertwig's general conception of "achromatic" structures in Protozoa, I believe that he has left out of account a number of facts which have an important bearing on the general problem. The "sphere" in *Noctiluca* is not the centrosome and must be distinguished from it; the centrosome in *Actinosphaerium* (Brauer) cannot be the same as the "pole-plate," and the "intra-nuclear" granule described by Schaudinn in *Amoeba*, and the so-called "centrosome" described by Balbiani must have some significance apart from the "achromatic" structures which accompany them. If we take these various structures into consideration, the problem becomes much more complex, and the possible differentiation of sphere (archoplasm) and centrosome must be sought for much earlier in phylogeny.

The most primitive nuclei in which a differentiated "achromatic" body occurs are found in *Euglena viridis* and *Amoeba crystalligera*. In both of these the nucleus consists of a nucleolus-like body with surrounding chromatin. Keuten ('95) describes this body, which up to this time had been called a "nucleolus,"¹ as similar to a nucleolus in its staining reactions, but as playing a different rôle in nuclear division. He therefore calls it a "nucleolus-centrosome."

My own observations on *Euglena viridis*, and on a species of *Cryptomonas* which has a similar central body, confirm every stage given by Keuten, but I give a different interpretation to the staining reactions of this so-called "nucleolus-centrosome." The color reactions which he describes for this body are not those of a centrosome. It becomes "orange-gelb" when stained with Orange G; with carmine solution it stains more intensely than the chromatin, while with haematoxylin it takes only a faint stain. In my preparations it takes a haematoxylin stain, remaining black or blue when the rest of the cell is stained with Orange G.

These reactions are characteristic of archoplasm or of the centrosphere, and for this reason, if for no other, the name "nucleolus-centrosome" is not entirely appropriate. Instead of comparing it with the centrosome of the Metazoa, it would be much more accurate to compare it with the sphere in *Noctiluca* or with the pole-plates of other Protozoa. A true centrosome has not been found in it.

¹ Considerable confusion has arisen because of the indiscriminate use of the term "nucleolus" in connection with protozoan nuclei. It has been applied to the chromatin-masses (karyosomes) and to various "achromatic" structures.

A similar central nuclear body has been described by Schaudinn in *Amoeba crystalligera*. With high powers he was able to make out a certain alveolar ("wäbige") structure of the "nucleolus" ("achromatic body"), and, in addition, he occasionally found in it a granule or granules which stained with chromatin dyes. He included all of these granules in the nucleolus. The chromatin undergoes no preparatory changes before division, and the nucleus divides first, the deeply staining granules having meantime disappeared.

Schaudinn considers this a confirmation of F. E. Schultze's earlier description of the division of *Amoeba* as amitotic, although he seems to realize the significance of the nucleolus when he says: "Der als Nucleolus bezeichnete Theil des Kerns scheint bei Durchschnürung des Kerns, wie Fig. II b u. III b zeigen, die Hauptrolle zu spielen" (p. 1035). The presence of granules within the "nucleolus" is interesting, and may be taken as a possible indication of an early stage in the differentiation of a centrosome.

The intra-nuclear "achromatic body" plays a more important rôle in the nuclei of *Euglypha*, *Spirochona*, and *Kentrochona*. In the former the bodies at the poles of the spindle are considered by Schewiakoff the same as the "Polkörperchen" (centrosomes) of metazoan nuclei. Their history is not clearly made out, but his description seems to indicate a nuclear origin, though he himself draws a different conclusion. His account is as follows: "The polar cytoplasm develops radial rays ('Polstralen') which converge at the 'Polkörperchen' (pole-plate) in a small invagination of the nuclear membrane. At the same time spindle-fibers make their appearance inside of the nucleus, and he concludes, therefore, that the 'Polkörperchen' is derived from the pole-rays by the coalescence of the cyto-microsomes lying in them. There is no mistaking Schewiakoff's meaning; the pole-bodies are derived from the cytoplasmic granules. There are, however, some significant features in his account of the division which throw considerable doubt on this inference. In the first place, at the time when the spireme is well formed, and just before the formation of the 'Polkörperchen' the so-called 'nucleolus' disappears.

There is, in reality, no occasion for using it at all, for the meaning is much better expressed by the terms "chromatic body" (or karyosome) and "achromatic body."

In the second place, during the anaphase 'the chromatin-loops become reduced to thick threads and the 'Polkörperchen' is redrawn into the nucleus.' Finally, after the stage just described, the chromatin regains first its coarse and then its fine mesh-like structure, and the 'nucleolus' reappears in the nucleus. If the 'Polkörperchen' is withdrawn into the nucleus, and if the nucleolus reappears shortly afterwards, it is probable that the 'nucleolus' is derived from the pole-bodies. Also, if the 'nucleolus' is thus derived from the 'Polkörperchen,' and if it disappears when the new pole-bodies are formed, an equally possible inference is that the pole-bodies are actually derived from the 'nucleolus.'” There is reason, therefore, to believe that in *Euglypha*, also, the “achromatic bodies” are intranuclear in origin.

The nuclear division of *Spirochona* was first described by Hertwig ('77). Since then it has been described by a number of observers, Balbiani ('95) being the most recent. Hertwig describes the resting-nucleus as consisting distinctly of two parts, one finely granular, and the other homogeneous in structure. Balbiani ('95) has, in the main, confirmed Hertwig's description of the *Spirochona* resting-nucleus. He finds, however, that the two parts of the nucleus are separated by a fissure or “fente,” which he thinks is the “Kernspalt” of the ciliate macronuclei. The “homogeneous” part, which undoubtedly corresponds to the “achromatic body” of other Protozoa, is, therefore, within the nuclear membrane, but, as in *Noctiluca* and *Paramoeba*, it is completely separated from the chromatin. Hertwig describes a small central granule in the homogeneous part, while Balbiani's description shows that other structures of a fibrous nature are also present before this body appears. He finds in stained preparations that the achromatic part (which appears homogeneous during life) contains numerous short fibers which form a network. These fibers do not stain with ordinary chromatin dyes and become arranged in a certain definite manner about the “nucleolus” (central granule in the “homogeneous” or achromatic body), which appears later.¹

¹ “La partie dite homogène du noyau ne merite pas non plus cette qualification dans le noyau fixé par les reactifs. Elle aussi présente un continu filamenteux

The exact history of the achromatic body of *Spirochona* is not given either by Hertwig or Balbiani. The former says that at times of division the homogeneous part enlarges, and a small central granule, which he calls the "nucleolus," appears. This gradually changes by sending out amoeboid processes, after which it becomes more and more indistinct until it can no longer be made out. The granular chromatic portion breaks into smaller pieces until the entire nucleus appears homogeneous. After this two masses of homogeneous substance, which he calls the "end plates," appear at the two extremities, and these he regards as the same substance as the original homogeneous portion. Balbiani also thinks that the "end plates" or pole-plates (Calottes) are the same as the homogeneous part of the resting-nucleus.¹

In all the larger *Spirochona* nuclei the homogeneous part (*i.e.*, the "achromatic body") encloses that granule which Balbiani with Hertwig calls the "nucleolus." In smaller nuclei, on the other hand, an analogous body is found in the granular and not in the homogeneous part. Hertwig shows no connection between these two granules, but claims that the "nucleolus of the homogeneous part is the homologue of the metazoan nucleolus." Plate ('86) gives a somewhat different account, and admits a possible identity of the two. The "nucleolus" of the homogeneous part, he thinks, is formed from particles of chromatin which penetrate, while in solution, from the granular into the homogeneous portion, and there reform into the solid refringent corpuscle. Bütschli regards the corpuscle as a condensation of the chromatin-reticulum of

comme la partie granuleuse, mais les filaments sont plus courts, plus fins, beaucoup moins nombreux, et au lieu d'être placés parallèlement les uns aux autres, ils s'entrecroissent diversement dans la substance homogène et transparente (suc nucléaire) dans laquelle ils sont plongés. De plus ils ne se colorent pas, ou faiblement, par les colorants de la chromatine, notamment le vert de méthyle, et se montrent des lors comme formes d'achromatine. Cette structure de la partie homogène ne s'observe que dans les noyaux qui ne contiennent pas encore un nucléole (centrosome) ou dans ceux où le nucléole se trouve encore dans la partie granuleuse, lorsqu'il est parvenu dans la partie homogène les filaments achromatiques prennent une autre disposition que nous décrirons par la suite" (p. 248).

¹ "Nous pouvons donc conclure à une identité morphologique et chimique complète entre les masses polaires du noyau en division et la partie achromatique du noyau au repos" (p. 292).

the homogeneous portion. Balbiani agrees with neither. He thinks that the "nucleolus" which is found in the granular part of the nucleus is not an initial but a final step in division (telophase), the corpuscle or "nucleolus" forming as follows: The ends of all the chromatin filaments converge at the pole during the telophase. These ends, which at first are closely pressed together, separate later, and a space or vacuole is made containing several granules. The latter collect together at a later stage to form a single corpuscle. This corpuscle is the "nucleolus," and from here it passes through the granular and into the homogeneous part of the nucleus, where it undergoes the changes so carefully described by both Hertwig and Balbiani.

The peculiar changes which this corpuscle undergoes led Balbiani to regard it, quite independently of the "pole-plates," as the "centrosome."

He says: "Le globule central participe à la fois des caractères d'un nucleole vrai, et d'un centrosome; comme nucleole, il disparaît par resorption dans la substance achromatique au debut de la division, pour se regenerer chez les deux nouveaux noyaux par les processus indiqué plus haut; comme centrosome il condense autour de lui la substance environnante son forme d'une petite sphere attractive intra-nucleaire, que ne passe pas du noyau dans le protoplasma pour y jouer le rôle d'un centrosome ordinaire pendant la division de la cellule" (p. 309). He denies Hertwig's homology of "end plates" to "Polkörperchen" or centrosomes, because they are nuclear in origin, whereas the latter are cytoplasmic, and he attempts to explain them "comme des accumulations, aux deux poles du noyau en division, de sa substance achromatique, et leur destinée est de ramener les noyaux nouveaux, au type normal du noyau au repos chez le Spirochone" — which explains nothing at all.

From Balbiani's description of the "achromatic" parts in *Spirochona* it is evident that this nucleus presents one achromatic element separated from the chromatin by a "fente," and similar to the sphere in *Noctiluca*; and a second element, — the so-called "centrosome" — which is derived from the chromatic portion of the nucleus. This body passes from the chromatin into the "achromatic" part of the nucleus, possibly in the same manner as the centrosome in *Noctiluca* is supposed to pass from the nucleus into the sphere. It cannot be identified with

the "nucleolus-centrosome" of *Euglena*, for the latter is "achromatic" and probably equivalent to the pole-plates of *Spirochona*.

A very similar description has been given in the case of another parasitic ciliate, *Kentrochona Nebaliae*. According to Römpel's ('94) description, the nucleus of *Kentrochona* develops at one pole an "achromatic" mass, which extends outwards and bears two centrosomes at its extremity. After this mass is well developed a similar structure appears at the opposite pole.

Römpel then proceeds as follows: "Wie wird der Gegenpol gebildet? Genauer, in welchem Lageverhältniss stehen Chromatin und Kernspindel (achromatic portion) während der Ausbildung des Gegenpols? Nach den vorliegenden Präparaten dürften überhaupt nur zwei Möglichkeiten in Betracht kommen. Entweder wird das von Anfang an ring- oder cylinderförmige Chromatin von der Kernspindel central durchbohrt, und diese wird so zur Achse des Chromatinhohlcyinders, oder die Kernspindel zieht sich unter dem ventral eingebuchteten Chromatinhohlcyinder her." Römpel's Fig. 4 *e* represents the latter alternative, which certainly suggests the action of the sphere in *Noctiluca*; for in *Kentrochona* the "achromatic Kernspindel," like the "central-spindle" of *Noctiluca*, is apparently sunk in the nucleus and stretches from pole to pole.

Römpel's account of the division of *Kentrochona* is very faulty, and the different stages described are separated by wide gaps. His "centrosomes," too, have been questioned by Hertwig ('96), Balbiani ('94), Flemming ('94), and others, most of whom think he has mistaken micronuclei for centrosomes. Their criticisms are upheld by Doflein ('96, '97), who has recently reexamined mitosis in *Kentrochona*. According to him the process of division is very similar to that of *Spirochona*.

"Die Theilung des Hauptkerns stammt in den grossen Zügen mit derjenigen bei *Spirochona* überein, wie sie besonders eingehend R. Hertwig und Balbiani geschildert haben" (p. 363). The chromatin is sharply defined against the achromatin which "in seitlicher Ansicht erscheint sie als Anlage der Polplatte" (p. 364).

Doflein mentions a distinct granule in the pole-plate during the metaphase, which must be analogous to the so-called "nucleolus" or "centrosome" of *Spirochona* according to Balbiani.

In *Actinosphaerium* the centrosome has been described by only one observer, Brauer ('94), and its presence has been since denied by Hertwig ('96). Here as elsewhere, however, one positive observation by a good authority must bear more weight than a number of negative statements. Both Hertwig and Brauer are agreed that the pole-plates in *Actinosphaerium* are derived from the intra-nuclear "achromatic" body of the resting nucleus. Brauer describes an accumulation of cytoplasm outside of the pole-plates which he calls the cytoplasmic "Kegel." The nuclear membrane is never lost at any point, nor is there any apparent connection between the protoplasmic "Kegel" and the inside of the nucleus, for they are separated by the pole-plates which "machen den Eindruck, als wenn es Verdickungen der Membran wären, die stets während des ganzen Kerntheilungsprocesses erhalten bleibt" (p. 204). The cytoplasmic accumulation appears only during mitosis. "Ausserhalb des Kernes an seinen Polen beginnt sich schon, wenn die ersten Veränderungen im Kern erkennbar werden, feinkörniges Protoplasma anzusammeln" (p. 204). In certain cases centrosome and radiations were seen in this mass, and from Brauer's figures of these stages it appears that the pole-plates at this time are either absent (Figs. 44, 46) or else much reduced (Fig. 45). Brauer is inclined to think that the centrosome is within the pole-plate during prophase and metaphase, and that it finds its way thence into the protoplasm during the anaphase. "So scheint mir der Schluss unabweisbar, dass das Centrosom vorher nicht im Protoplasma ausserhalb des Kernes, wie die Figuren es zeigen, gelegen haben kann" (p. 207).

Thus in *Actinosphaerium* the substance of the pole-plates, or a part at least, becomes extra-nuclear for a short time. In *Paramoeba* (Schaudinn, '96) and in *Noctiluca* it is permanently so. Schaudinn describes it in *Paramoeba* as follows: "Dicht neben dem Kern liegt stets das bereits zu Anfang erwähnte, stark lichtbrechende, scharf construirte Gebilde. Ich will dasselbe zunächst mit einem ganz indifferenten Namen, etwa als 'Nebenkörper' bezeichnen. Bei den kleinsten Amöben ist es kugelig und ungefähr von derselben Grösse wie der Kern." During spore-formation, *pari passu* with a progressive

division of the nucleus, this "Nebenkörper" breaks up into smaller masses, each of which becomes associated with one of the daughter-nuclei. Here it forms a central-spindle, and the spore-nuclei divide by mitosis, in the same way, apparently, as in *Noctiluca*. A centrosome is not described.

A review of the foregoing facts shows that different forms of Protozoa present a nearly complete series in which may be traced the possible development of the complex extra-nuclear centrosome and sphere from an intra-nuclear "achromatic" body. This, — the homologue of the attraction-sphere, — apparently, first appears as an axial nuclear rod in *Amoeba crystalligera* and *Euglena viridis*. In *Spirochona*, *Kentrochona*, and the micronuclei of Infusoria it is seen as aggregations of "achromatin" — pole-plates — at the spindle poles. In *Actinosphaerium* the pole-plates remain intra-nuclear for the greater part of mitosis, but according to Brauer a portion of them at least becomes extra-nuclear for a brief period; and during this period spindle-fibers are formed. The "achromatic" body remains extra-nuclear in *Paramoeba*, in *Noctiluca*, and in the majority of Metazoa. In *Noctiluca* and the Metazoa a portion of the "achromatic" body, — the central-spindle, — although extra-nuclear, comes to lie during mitosis in the center of the nuclear plate, where it occupies the same position, morphologically, that it occupies in *Amoeba crystalligera* or *Euglena viridis*.

The centrosome is not so easily traced, and it may be found that in the majority of Protozoa the intra-nuclear so-called "centrosome" is quite a different structure from the centrosome of the Metazoa, although even here the centrosome has been traced to an intra-nuclear position by many observers. Its nuclear origin in Protozoa is made out by Balbiani in *Spirochona* and by Doflein in *Kentrochona*, while Schaudinn describes a body in *Amoeba crystalligera* which must be homologous. In one case at least — *Spirochona* (Balbiani) — its direct morphological connection with the chromatin has been traced, although not conclusively.¹

¹ In this case the centrosome relation can be questioned on account of the place of origin of the supposed centrosome. In most cases the centrosome is found in the region of the spindle poles, but in *Spirochona*, according to Balbiani,

In *Spirochona* the so-called "centrosome" passes from the nuclear chromatin into the achromatic body, which, however, still lies within the nuclear membrane. It is possibly nuclear in origin in *Actinosphaerium*, becoming extra-nuclear during the metaphase of division. It appears to be nuclear in origin in *Noctiluca*, but is found in the cytoplasm in the achromatic body in the early metaphase and remains permanently in the cytoplasm in spore-forming individuals. Finally it is permanently cytoplasmic in the Metazoa, although occasionally nuclear in origin even here (*Ascaris megalocephala univalens*).

The important position that *Noctiluca* must hold in all theories of mitotic development has not hitherto been sufficiently emphasized. Much stress, indeed, has been laid on its similarity in mitosis to metazoan cells, but little has been done to show its relations to other Protozoa and the origin of its mitotic structures. When considered alone, *Noctiluca* can throw but little light on the origin of the complex elements of the metazoan mitotic-figure, but when considered in connection with other Protozoa the origin of its own elements is seen, and so, indirectly, the probable origin of the elements in Metazoa. *Noctiluca* thus holds an intermediate position in the probable development of mitosis, and the inference may be drawn that the origin of the mitotic elements in Metazoa is the same as in *Noctiluca*.

The position which *Noctiluca* holds in the development of chromatic structures has been sufficiently pointed out. It has been shown, first, that *Noctiluca* has probably the most primitive form of true chromosomes; and, second, that *Noctiluca* presents probably the most primitive ring-like arrangement of the chromosomes and nuclear plate around the central-spindle. In regard to the "achromatic" structures the position of *Noctiluca* is not quite so definite, and to be understood must be considered in connection with lower forms. It has been mainly originates from chromatin in the region of the "Zwischenkörper." Further work on this questionable structure must be done before its proper position can be determined; that it is an important element in the cell and has a rôle to play in nuclear division is established beyond question by the independent observations of Hertwig, Plate, Bütschli, and Balbiani.

tained by numerous observers that the so-called "achromatic structures" of the mitotic-figure are primitively nuclear in origin. (See O. and R. Hertwig ('93 and '96), Heidenhain ('94), etc.) Furthermore, in the various kinds of Protozoa which have been carefully studied the "achromatic" portions of the mitotic-figures are developed from permanent, definite bodies which are independent of the chromatin, although contained within the cell-nucleus. As shown elsewhere in this paper, two apparently similar elements of the metazoan cell have been described, either or perhaps both of which may be comparable to these intra-nuclear achromatic bodies in Protozoa. The first of these is the "archoplasm" of Boveri; the second is the substance which forms the "centrodesmus" of Heidenhain, which is described as a specific substance of the cell, as playing a certain definite rôle in both resting and active phases, and as distinct from the centrosome, from the nucleus, and from the cytoplasm. Whether we consider this substance in the form of "centrodesmus" or as archoplasm it appears, therefore, that the cells of some Metazoa have a permanent specific substance which may be considered the homologue of the "achromatic body" of Protozoa.

Boveri does not consider a nuclear origin contrary to the conception of archoplasm, and with many others regards the "achromatin" in many protozoan nuclei as archoplasm ('97). He even holds it possible that there may be nuclei in Metazoa which show a return to this primitive condition. Heidenhain does not mention the derivation of the "centrodesmus" from any other structure in more primitive forms. The only standards of identification are: its relation to the centrosome, the formation of the spindle from its substance, and its permanency. These same points may be used equally well for determining the more primitive forms of archoplasm.

In *Noctiluca* the so-called "sphere" surrounds the centrosome at the period when the latter is unmistakable. Here, also, the central-spindle is formed from the substance of the sphere, and the latter is permanent in the cell, persisting as a specific substance throughout all stages and quite distinct from the cytoplasmic reticulum. The sphere in *Noctiluca*, therefore,

appears to be equivalent to the archoplasm of Boveri and the centrodesmus of Heidenhain. In the more primitive forms, unfortunately, the behavior of the intra-nuclear "achromatin" during division is not well enough known to warrant definite conclusions, although the fragmentary evidence which has been gathered from various sources is sufficient to show, I believe, that structures possessing all of the attributes of archoplasm are present in the various forms. Its history is well known, indeed, in *Euglena*, but here we are confronted with the assumption, by Keuten and others, that the intra-nuclear element is a centrosome or its equivalent. There is reason to believe, however, that the two poles of this achromatic body represent the pole-plates of other Protozoa, and that the connecting rod represents the central-spindle. If this hypothesis is correct, the achromatic body in the nucleus of *Euglena* must be considered equivalent to the sphere in *Noctiluca* and to the archoplasm of Boveri or the centrodesmus of Heidenhain.

With our present knowledge it is impossible to go farther back than *Euglena* for the development of archoplasm, and the conclusion which may finally be drawn from our present knowledge of this difficult question seems to be that primarily there was a specific substance of the cell (archoplasm in Boveri's sense) connected in some way with the mechanism of cell division, and forming a definite intra-nuclear body (*Euglena*). Secondly, that this body became permanently extra-nuclear, but still connected with nuclear division (*Noctiluca*, and Metazoa with "centrodesmus"), and finally that it became lost in the cell and indistinguishable from the cytoplasmic reticulum (cells without archoplasm or centrodesmus, most egg cells).

The close similarity of mitosis in *Noctiluca* and Metazoa does not necessarily indicate any phylogenetic connection. Nor do the various Protozoa, which in this analysis are necessarily brought together, show phylogenetic characters. We are at present unable to develop any phylogenetic theory from the facts of nuclear division. All that can be maintained is that mitosis, in its many complicated phases, may have passed through stages of development which are to-day represented by many different and unallied types of Protozoa.

IV. SUMMARY OF OBSERVATIONS.

1. The resting nuclei of *Noctiluca miliaris* are large, round, or oval structures, containing (a) chromatin in the form of karyosomes, and (b) "achromatin" in the form of large granules. It is enclosed by a nuclear membrane which persists in part throughout nuclear division.

2. A cytoplasmic substance, corresponding to the centrosphere of many metazoan cells, is invariably present. It is a permanent organ of the cell, often as large, or larger, than the nucleus; it divides to form an amphiaster, consisting of two asters with connecting mantle-fibers, the central-spindle.

3. During the division of the sphere the nucleus elongates and bends to form a figure like the letter C; the central-spindle sinks into the opening thus formed, and is finally almost enclosed by the nucleus.

4. As division progresses, the central-spindle becomes three or four times as long as it is in the metaphase.

5. The karyosomes break up, by repeated division, into innumerable chromatic elements. In some cases beaded fibers are formed by the linear arrangement of larger chromatin particles. These fibers are the only indication in *Noctiluca* of a spireme-stage. The chromatin elements begin to form the chromosomes, which, at first, are lines of single granules extending from the nuclear membrane on the side next the sphere towards the opposite side. At this stage they appear like radial-fibers extending around the central-spindle and forming a nearly closed ring. This incomplete ring of chromosomes is the nuclear plate.

6. The chromosomes next become thicker, especially at the ends next the central-spindle, and, probably, by the aggregation and fusion of the granules. This enlargement continues towards the opposite ends, until, finally, the chromosomes are of uniform thickness.

7. The chromosomes divide longitudinally and while lying in the nuclear plate. The halves then separate, beginning at the proximal ends.

8. While the chromosomes are forming, the nuclear membrane disappears from between the chromosomes and the central-spindle. This leaves the chromosomes in contact with the spindle-fibers.

9. The central-spindle fibers have no connection with the chromosomes, but pass without interruption from pole to pole. The chromosomes are connected with the spheres by another set of fibers, — the mantle-fibers, — which pass from centrosomes in the spheres to the ends of the chromosomes.

10. The nucleus, during the anaphase, elongates in the direction of the central-spindle. The chromosomes are pulled apart, the final division taking place at the distal end of each. As they separate still more, they form two sets of oppositely-directed striations in the nucleus, and the daughter-chromosomes again thicken at the proximal ends.

11. The nucleus, finally, divides in the center, often with a very large connecting-piece between the daughter-nuclei. The furrow is obliterated, the nuclear membrane reforms, and the nucleus rounds out. The sphere loses its densely compact appearance and becomes more expanded, although its granular structure is retained.

12. In spore-forming divisions the nuclei do not return to the resting state. The daughter-spheres divide and form secondary or tertiary, etc., amphiasters; the daughter-chromosomes form the nuclear plate of the next mitosis without change; and split again longitudinally. This process is repeated eight or nine times.

13. A centrosome is always found in the sphere during metaphase and anaphase stages as the focal point of the mantle-fibers, but is not found during resting stages. It divides in the early anaphase in anticipation of the next mitosis.

14. The centrosome, possibly, comes from the nucleus, where, during the resting stages, a small, deeply staining granule can be easily distinguished from the chromatin. This granule disappears during the early stages of chromosome-formation. At the same period the nuclear membrane, just below the sphere, shows distinct undulations, or wrinkles, which form a clear space below the membrane. In numerous

cases two distinct granules were seen in this space. These granules occupy various positions in relation to the membrane, and in some cases they were found outside of the nucleus and in the sphere. The inference is therefore drawn that the centrosome is a permanent cell organ, which is not found in the resting sphere, but in the nucleus, from whence it becomes extra-nuclear by a rupture in the nuclear membrane. The observations on this head are not conclusive, owing to the smallness of the objects and to the presence of many similar cytoplasmic microsomes.

COLUMBIA UNIVERSITY,
November, 1897.

APPENDIX.

Since the above was written a number of forms have been carefully examined in the hope of finding Protozoa in which the archoplasm may be traced back to a type still more primitive than *Euglena viridis*. It was found that *Trachelomonas hispida*, *T. volvocina*, *Microglena punctifera*, and *Synura uvella* have nuclei similar to that of *Euglena viridis*; i.e., with a central body and chromatin in the form of small granules. In another set of forms, including *Trachelomonas lagenella*, *T. hispida* (variety), and *Chilomonas cylindrica*, the chromatin is in the form of granules and, as in *Euglena*, surrounds a central body, but, unlike *Euglena*, there is no nuclear membrane. Finally, in a species of *Tetramitus*, not only is the nuclear membrane absent, but the granular chromatin is distributed throughout the cell, and only during division are the granules collected around a central body. This central body corresponds evidently to the Nebenkörper of the flagellate-stage of Schaudinn's *Paramoeba Eilhardi*, but in *Tetramitus*, except during cell division, it is cytoplasmic, no morphological nucleus being present. The relations of this body to the chromatin are the same as the relations of the sphere to the chromatin in *Noctiluca*, although the former is far more primitive because of the distributed nucleus (cf. Bacteria). It would seem, therefore, that archoplasm was originally cytoplasmic; that it attracts (?) the chromatin about it during cell division (*Tetramitus*); that, in somewhat

higher forms, its attractive force keeps the granules together during resting phases as well as during division (*Chilomonas*, *Trachelomonas*, etc.); and, finally, that in still higher forms a nuclear membrane is formed about the whole (*Euglena*, *Trachelomonads*, *Synura ciliates*, etc.). But another line of development may have arisen from this primitive type. In some forms the archoplasm may have remained outside of the aggregate of chromatin granules, becoming secondarily centralized in the manner described by Schaudinn ('96) for the division of the flagellate-stage of *Paramoeba*, or in the same way as the central-spindle in *Noctiluca* becomes centralized. In this way the sphere in metazoan cells may have arisen without any connection with the intra-nuclear body of most Protozoa.¹

May 24, 1898.

¹ G. N. Calkins, The Phylogenetic Significance of Certain Protozoan Nuclei. *Ann. N. Y. Acad. Sci.*, vol. xi, no. 16, 1898.

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DESCRIPTION OF PLATES.

Figs. 11, 16-19, 21, 22, 24, 26-31, 38 represent nuclei in the process of spore-formation; Figs. 1-10, 12-15, 20, 23, 25, 32-37, 39 are various stages of the vegetative nucleus. Picro-acetic was used for individuals represented in Figs. 1, 5, 8, 15, 19, 20, 24; sublimate-acetic (weaker solution) for Figs. 6, 7, 10, 13, 17, 18, 21-23, 25, 32-37, 39. Stronger solution for Figs. 26, 27, 29-31, 38; Hermann solution for Figs. 9, 11, 12, 14, 16, 28; and corrosive sublimate for Figs. 2, 3, 4. The iron haematoxylin was used in all cases save Fig. 10, where the Biondi-Ehrlich mixture was used. Figs. 1-9, 11-18, 20, 21 are from preparations *in toto*; Figs. 10, 19, 22-38, from sections. x = supposed intra-nuclear centrosome.

FIG. 1. Resting stage of a vegetative nucleus showing 9 karyosomes and granular oxychromatin. Sphere on the outside of the nuclear membrane presents characteristic granular cortex and hyaline central part.

FIG. 2. Nucleus showing early stages in fragmentation of the karyosomes.

FIG. 3. Nucleus showing possible method of karyosome fragmentation. x = the supposed intra-nuclear centrosome.

FIG. 4. Later stage in karyosome fragmentation. The granules are gathered in groups, each group representing a previous karyosome.

FIG. 5. A still later stage in karyosome fragmentation. The sphere appears homogeneous.

FIG. 6. A nucleus in the so-called "spireme-stage." The chromatin granules are still large, but are arranged in fibers. The supposed centrosome (x) is distinct from the chromatin.

FIG. 7. Same as Fig. 6.

FIG. 8. A nucleus in the prophase of division. The achromatin granules are arranged in fibers parallel with the chromosomes. The chromosomes are forming with the thickened ends towards the sphere. The sphere has become more dense, and the hyaline center is disappearing. Some of the karyosomes are not yet fragmented.

FIG. 9. A prophase of division. The granules are beginning to form fibers, — the chromosomes. The sphere is dividing. Numerous processes stretch out from the sphere into the cytoplasm. These are probably equivalent to astral fibers.

FIG. 10. The same stage in section. The nucleus is filled with granules which are forming chromosomes.

FIG. 11. Stage of the amphiaster. The chromosomes are all formed and are represented in optical section in the nuclear plate.

FIG. 12. An abnormal nucleus. The sphere is bent into an acute angle. The nucleus shows elongation in the primary axis before chromosome-formation.

FIG. 13. The nucleus and amphiaster during metaphase. The latter lies in the secondary axis. Central-spindle, nuclear plate, and asters are shown. Mantle-fibers not represented.

FIG. 14. Metaphase showing position of the nuclear plate and the dumb-bell-shaped amphiaster.

FIG. 15. Metaphase stage viewed from the opposite side, *i.e.*, the side away from the central-spindle. The chromosomes are seen in optical section.

FIG. 16. Late anaphase seen from side opposite the spindle. The chromosomes are completely separated and show secondary thickenings at the ends towards the spheres. The nucleus has elongated in the direction of the secondary axis.

FIG. 17. Late anaphase showing the position of the central-spindle; the chromosomes are completely separated.

FIG. 18. Telophase showing the formation of the secondary nuclear plates and just previous to the division of the daughter-spheres. The plane of the next division is therefore clearly indicated.

FIG. 19. Late anaphase showing final division of the chromosomes. The nucleus and the central-spindle were curved so that only one pole (the upper) was sectioned.

FIG. 20. Late anaphase in vegetative division. The chromatin of the chromosomes is reforming into large karyosomes. The daughter-spheres are beginning to expand and to lose their densely granular appearance, while the characteristic hyaline portion of the central part is plainly indicated, resembling the sphere in Fig. 1.

FIG. 21. Late telophase and beginning of the secondary divisions. The daughter-spheres have formed amphiasters, although the parent nucleus is not completely divided as shown by the large "Verbindungsstück."

FIG. 22. Section of nuclear-plate stage showing nuclear plate and fan-like arrangement of the chromosomes.

FIG. 23. Late anaphase showing the collection of chromatin into karyosomes. A centrosome (*C*) is plainly indicated at the focus of the mantle-fibers. There is no nuclear membrane between chromosomes and sphere.

FIG. 24. Similar anaphase showing aggregation of daughter-chromosomes as in Fig. 18. No karyosome-formation taking place. Centrosome and mantle-fibers present.

FIG. 25. Late telophase in vegetative division. Remnants of the daughter-chromosomes can still be made out, although most of the chromatin is now collected in a number of karyosomes.

FIG. 26. Transverse section showing the double nature of the chromosomes and compact arrangement of the proximal ends.

FIG. 27. Similar section showing one chromosome (*A*) beginning to split at the proximal end.

FIG. 28. Later stage. Mantle-fibers connect double centrosomes with the dividing chromosomes. Two separate bundles of daughter-chromosomes can already be made out.

FIG. 29. Division of chromosomes is carried still farther; the distal ends are not yet divided. In both figures the characteristic crossed appearance of this stage is plainly visible. The nuclear membrane persists except where mantle-fibers connect centrosome and chromosomes.

FIG. 30. A later stage in chromosome division. The distal ends are now separated. The nuclear membrane has reappeared between the central-spindle and the chromosomes.

FIG. 31. Horizontal section of similar stage. The central-spindle lies through the middle of the figure, the nuclear membrane is intact except at the poles. This is about the same stage represented in Fig. 17, and would represent a section cut in the plane of the paper.

FIG. 32. Prophase of vegetative division showing slight undulation in the membrane below the sphere, a hyaline space below the undulation, and two distinct granules in the hyaline space.

FIG. 33. Prophase of vegetative division showing very marked undulation of the membrane below the sphere, a distinct hyaline sphere, and again two granules within the space.

FIG. 34. Prophase of vegetative division. A distinct depression is now formed at the point where undulations appeared in the other cases. The two distinct granules are now seen in the space just outside of this depression.

FIG. 35. Prophase of vegetative division. The undulations are very marked in the region of the sphere, and are not found elsewhere. The two distinct granules are outside of the membrane.

FIG. 36. A similar stage. The two granules now lie in the sphere, the chromatin is in the so-called spireme-stage, and chromosomes are forming.

FIG. 37. The two granules are now very distinct and lie in the sphere. The nucleus is elongated in the primary axis, but the chromatin is still widely distributed.

FIG. 38. Section through primary axis of a daughter-nucleus in about the same stage as that shown in Fig. 18. The chromosomes are double, the mantle-fibers are distinctly granular, and the centrosome is double. The sphere has not yet divided.

FIG. 39. Late anaphase in vegetative division. The nuclear membrane is not yet reformed, the mantle-fibers are disappearing, and the chromosomes are disintegrating.





